UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

DATE: November 20, 2007

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Secondary Review of Contractor's (DynCorp Systems & Solutions LLC) Efficacy Review for DisCide ULTRA Disinfecting Towelettes;
EPA Reg. No. 10492-4;
DP Barcode: D344274

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APPLICANT: Palmero Health Care
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FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
n-alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride	0.12%
n-alkyl (68% C12, 32% C14) dimethyl ethyl benzyl ammonium chloride	0.12%
Isopropyl alcohol	63.25%
Other Ingredients	36.51%
Total	100.00%

I BACKGROUND

The product, DisCide ULTRA Disinfecting Towelettes (EPA Reg. No. 10492-4), is an Agency approved disinfectant towelette (bactericide, tuberculocide, virucide, fungicide) for use on hard, non-porous surfaces in institutional, commercial, food service, animal care, and hospital or medical environments. The applicant requested to amend the product registration to add claims for effectiveness as a disinfectant against Avian Influenza (H3N2) virus, Influenza A virus, and Respiratory syncytial virus. The label claims that the product is effective in "one-step" (i.e., effective in the presence of organic soil). Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; and at MicroBioTest, Inc., located at 105 Carpenter Drive, in Sterling, VA 20164.

This data package contained a letter from the applicant's representative to the Agency (dated August 6, 2007), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), three studies (MRID 472019-01 through 472019-03), Statements of No Data Confidentiality Claims for all three studies, the proposed label, and the last-accepted label (dated October 4, 2005).

Note: EPA Form 8570-4 (Confidential Statement of Formula) contains Confidential Business Information. Data or information claimed by the applicant to be FIFRA confidential has not been included in this report.

II USE DIRECTIONS

The product is designed for use in disinfecting hard, non-porous, non-food contact surfaces such as appliance exteriors, baskets, bassinets, bathroom fixtures, bed rails, cabinets, carts, cell phones, chairs, child-restraint seats, computer keypads, computer screens, counter tops, diaper pails, drawer pulls, door knobs and handles, exercise and weight-lifting equipment, flooring, incubators, lighting fixtures, office equipment, physical therapy equipment, shopping carts, shower stalls, sinks, stools, tables, telephones, toilet exteriors, toilet seats, trash cans, vanities, and walls. The label indicates that the product may be used on hard, non-porous surfaces including: Formica, glass, and stainless steel. Directions on the proposed label provided the following information regarding use of the product as a disinfectant against viruses: Thoroughly wet the surface with the towelette product. Allow to remain wet for 1 minute.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Antimicrobial Products for Use on Hard Surfaces Using Pre-saturated or Impregnated Towelettes

Towelette products represent a unique combination of antimicrobial chemical and applicator, pre-packaged as a unit in fixed proportions. As such, the complete product, as offered for sale, should be tested according to the directions for use to ensure the product's effectiveness in treating hard surfaces. The standard test methods available for hard surface disinfectants and

sanitizers, if followed exactly, would not closely simulate the way a towelette product is used. Agency guidelines recommend that a simulated-use test be conducted by modifying the standard test methods. Agency guidelines further recommend that instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the slides after a specified holding time. Performance standards of the standard test methods must be met. These Agency standards are presented in EPA Pesticide Assessment Guidelines, Subdivision G, §91-2(h), Pre-saturated or impregnated towelettes; and the April 12, 2001 EPA Memorandum, Draft Interim Guidance for Non-Residual Sanitization of Hard Inanimate Food Contact Surfaces Using Pre-Saturated Towelettes.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Supplemental Claims

An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. These Agency standards are presented in DIS/TSS-2.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 472019-01 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus," for DisCide Ultra Disinfecting Spray, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – July 18, 2007. Project Number A05114.

This study was conducted against Influenza A virus (Strain Hong Kong; ATCC VR-544) using cultures of Rhesus monkey kidney (RMK) cells (obtained from Viromed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 14-05257B

and 14-04187C) of the product, DisCide Ultra Disinfecting Spray, were tested according to ATS Labs Protocol No. PAL02042707.FLUA (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 52% relative humidity. For each lot of product, one dried virus film was sprayed (10 sprays) with the product at a distance of 6-8 inches from the carrier surface. Each virus film was exposed to the product for 1 minute at 20.0°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 8 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

2. MRID 472019-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A (H3N2) virus (Avian Reassortant)," for DisCide Ultra Disinfecting Spray, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – July 18, 2007. Project Number A05112.

This study was conducted against Avian Influenza A (H3N2) virus (Avian Reassortant; ATCC VR-2072; Strain A/ Washington/897/80 X Mallard/New York/6750/78) using cultures of Rhesus monkey kidney (RMK) cells (obtained from Viromed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 14-05257B and 14-04187C) of the product, DisCide Ultra Disinfecting Spray, were tested according to ATS Labs Protocol No. PAL02042707.AFLU (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 52% relative humidity. For each lot of product, one dried virus film was sprayed (10 sprays) with the product at a distance of 6-8 inches from the carrier surface. Each virus film was exposed to the product for 1 minute at 20.0°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 8 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls

included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

3. MRID 472019-03 "Virucidal Efficacy Test, Respiratory Syncytial Virus" for DisCide Ultra Disinfecting Spray, by Lisa M. Lundberg. Study conducted at MicroBioTest, Inc. Study completion date – March 9, 2006. Laboratory Project Identification Number 552-111.

This study was conducted against Respiratory syncytial virus (ATCC VR-26) using cultures of HEp-2 cells (ATCC CCL-23) as the host system. Three lots (Lot Nos. 14-06205A, 14-08235A, and 14-11085A) of the product, DisCide Ultra Disinfecting Spray, were tested according to a MicroBioTest protocol titled "Virucidal Efficacy Test, Respiratory syncytial virus," dated September 25, 2005 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, one dried virus film was sprayed with the product at a distance of 12 inches from the carrier surface, until thoroughly wet. Each virus film was exposed to the product for 1 minute at 21°C. After exposure, 2.0 ml of newborn calf serum was added to each plate to neutralize. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI 1640 containing 5% newborn calf serum. HEp-2 cells in culture dishes were inoculated in quadruplicate with an unspecified amount of each dilution. The cultures were incubated for an unspecified amount of time at 37±1°C for viral adsorption. Post-adsorption, the cultures were re-fed with test medium and incubated for 10-14 days at 37±1°C in 5±1% CO_2. Post-incubation, the cultures were examined for the presence of residual infectious virus. Controls included those for cell viability/ sterility, plate recovery count, cytotoxicity, cytotoxicity-related viral interference, and neutralizer effectiveness. The 50% cell culture infectious unit dose per ml ($CCID_{50}$/ml) was determined using the method of Reed and Muench.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The laboratory report includes a "Confidentiality" clause on page 24, which restricts the reporting of data to the public.

V RESULTS

MRID Number	Organism	Results			Dried Virus Control ($TCID_{50}$/0.1 ml)
			Lot No. 14-05257B	Lot No. 14-04187C	
472019-01	Influenza A virus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{6.25}$
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 ml	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 4.75 $\log_{10}$	≥ 4.75 $\log_{10}$	
472019-02	Avian Influenza A (H3N2) virus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{4.75}$
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 ml	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 3.25 $\log_{10}$	≥ 3.25 $\log_{10}$	

MRID Number	Organism	Results				Plate Recovery Control ($CCID_{50}$/ml)
			Lot No. 14-06205A	Lot No. 14-08235A	Lot No. 14-11085A	
472019-03	Respiratory syncytial virus	10^{-2} dilution	Cytotoxicity			$10^{5.77}$
		10^{-3} to 10^{-7} dilutions	Complete Inactivation			
		$CCID_{50}$/ml	$10^{2.50}$	$10^{2.50}$	$10^{2.50}$	
		Log reduction	3.27 $\log_{10}$			

VI CONCLUSIONS

1. The submitted efficacy data support the use of the liquid, non-towelette product, DisCide ULTRA Disinfecting Spray, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute:

Avian Influenza A (H3N2) virus	MRID 472019-02
Influenza A virus	MRID 472019-01
Respiratory syncytial virus	MRID 472019-03

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was observed in the 10^{-1}

dilutions for studies against Avian Influenza A (H3N2) virus and Influenza A virus. Cytotoxicity was observed in the 10^{-2} dilutions for studies against Respiratory syncytial virus. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

Note: For the two studies conducted at ATS Labs, virus films were sprayed with "10 sprays" of the product. It seems as if this number of sprays would deliver an excess (and unrealistic amount) of product, and this application instruction is not included on the proposed label. Often, 3-5 sprays are used.

VII RECOMMENDATIONS

1. The proposed label claims that the product, DisCide ULTRA Disinfecting Towelettes, is an effective "one-step" disinfectant against the following microorganisms on hard, non-porous surfaces for a contact time of 1 minute:

Avian Influenza A (H3N2) virus
Influenza A virus
Respiratory syncytial virus

Data provided by the applicant support these claims. Acceptable efficacy results (captured in an Agency letter dated October 4, 2005) against Adenovirus Type 2 virus (a non-enveloped virus, and the most resistant virus listed on the label), allow for bridging of susceptible, enveloped viruses to the proposed label.

2. To satisfy DIS/TSS-15 labeling requirements, please insert the following step in the directions for use: "Remove gross dirt and soil." or alternatively "Pre-clean heavily soiled surfaces prior to treatment." As noted in DIS/TSS-12, the suggested 5% organic soil load is considered appropriate for simulating lightly or moderately soiled surface conditions. Testing in the presence of a 5% organic soil load does not demonstrate efficacy in the presence of heavily soiled surfaces. As a result, surfaces that are heavily soiled must be pre-cleaned.

3. The following changes are required on the proposed label:

- On page 1 of the label, change "Respiratory Syncitial Virus" to read "Respiratory Syncytial Virus."
- On page 1 of the label, change "*Salmonella choleraesuis*" to read "*Salmonella enterica*."
- ATCC numbers are required in one of the following locations:
  - On the data matrix

- On the master label (as optional text) with the listing of the organisms claimed, or
- As the final page of the master label (as optional text)

- On page 2 of the label, under the "Special Instructions for Cleaning and Decontamination Against HIV-1 …" section, change "Allow to air dry." to read "Allow surfaces to remain wet for 1 minute, then air dry." It is important that surfaces remain wet for the duration of the contact time.